Spin Trapping of Protein Thiyl Radicals: EPR Analysis of Thiyl Radicals in R1 Protein of Ribonucleotide Reductase and in BSA

Günter Lassmann[1,2], Matthias Kolberg[2], Günther Bleifuss[2], Friedhelm Lendzian[2], Britt-Marie Sjöberg[3], Astrid Gräslund[4], and Wolfgang Lubitz[1]

[1]Max-Planck-Institute of Radiation Chemistry, Muelheim/Ruhr, [2]Max-Volmer-Laboratory of Biophysical Chemistry, Technical University of Berlin, Berlin, Germany, [3]Department of Molecular Biology, and [4]Department of Biochemistry and Biophysics, Arrhenius Laboratories, Stockholm University, Stockholm, Sweden

Ribonucleotide reductases (RNR) are metal-enzymes which utilize protein-radicals in a unique manner for their catalytic function. Protein thiyl radicals (R–S$^{\bullet}$) have been postulated as catalytically essential intermediates in all classes of RNR, but so far there is no spectroscopic evidence for their existence in the most studied enzyme, the aerobic *E.coli* RNR. We report for the first time on spin trapping detection and direct EPR detection of protein thiyl radicals in R1 protein of RNR.

Thiyl radicals have been artificially generated in the R1 protein of *E.coli* RNR which contains catalytically active thiols, and in the model protein bovine serum albumin (BSA) by two different methods. Protein thiols have been oxidized, (i) chemically using Ce^{IV}/nitrilotriacetate, and (ii) photochemically by photorelease of nitric oxide (NO) from nitrosylated thiols using a 355 nm laser beam. With both methods, spin adducts selectively from protein thiyl radicals has been observed by EPR spin trapping at room temperature using phenyl-N-t-butylnitrone (PBN). The EPR lineshape of the protein-bound spin adduct is typical for slow motion of the nitroxide moiety and indicates that the majority of trapped thiyl radicals are localized in a folded region of R1 [1]. The mild photochemical generation of protein thiyl radicals might open a way for futural mimickíng of suitable partial enzyme turnover steps to evidence their catalytic competence in RNR.

In aerobic R1 samples, without the spin trap PBN, frozen after exposure to the laser beam or after treatment by Ce^{IV}/NTA, sulfinyl radicals (R–S$^{\bullet}$=O) were observed by direct EPR detection and assigned via their g-tensor components. Sulfinyl radicals are the reaction product of thiyl radicals with oxygen and give additional evidence for generation of thiyl radicals in R1 by the procedures used [1].

Furthermore, we report on directly acquired EPR data, as g-tensor, line shape, saturation and stability, of protein thiyl radicals in frozen solution generated by UV irradiation at 77 K of R1 and BSA (without spin trapping) [2].

[1]. Matthias Kolberg, Günther Bleifuss, Britt-Marie Sjöberg, Astrid Gräslund, Wolfgang Lubitz, Friedhelm Lendzian, and Günter Lassmann, *Arch. Biochem. Biophys. (in press),*

[2]. Matthias Kolberg, Günther Bleifuss, Astrid Gräslund, Britt-Marie Sjöberg, Wolfgang Lubitz, Friedhelm Lendzian, and Günter Lassmann, *J. Amer. Chem. Soc. (submitted)*